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A micro E-DNA sensor for selective detection of dopamine in presence of ascorbic acid*

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In this paper, a novel method for selectively detection of dopamine (DA) in the interference of ascorbic acid (AA) is described. A nanometer-sized gold flower microelectrode (NGFME) is prepared by flame-etching and electrochemical deposition. The electrode tip was characterized by scanning electron microscope (SEM). The NGFME is sized at about 100 μm and dimensions of thorns of the electrode were in nanometers. By modifying with DA aptamer on the surface, the prepared aptasensor can selectively detect DA even in the presence of high concentration AA. Experimental results show that this NGFME has no response to AA. As a comparison, the carbon fiber electrode without DA aptamer modification is unable to effectively detect DA in the presence of AA. The NGFME is easy-to-prepare, selective and sensitive for DA detection down to 25 μM. The electrode can be expected to detect DA *in vivo* and in real biological samples.

Keywords: Electrochemical sensor, Microelectrode, Dopamine detection, Ascorbic acid

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I. INTRODUCTION

Dopamine (DA), a crucial catecholamine neurotransmitter molecule in neuropharmacology, is closely related to activities of human and animals [1, 2]. In the nervous systems, DA functions as a chemical release between nerve cells to send signals. The DA concentration in brain affects the emotion and behavior of human beings. Several diseases are associated with damage to the DA system, such as Parkinson's disease. Therefore, fast and quantitative DA monitoring *in vivo* is strongly desired. However, in tissues of mammals, DA is always coexisting with other substances, such as ascorbic acid (AA) [3–5], a vitamin maintaining physiological function of the body. Tissue AA concentrations are 100–1000 folds higher than DA, overlaping oxidation potential of DA and disturbing DA detection [6–8]. So, selectively detecting DA from AA interference has practical meaning in clinical diagnosis.

A sensitive and convenient way of DA detection is the use of electrochemical sensors [4–10]. The electrode for DA detection *in vivo* should be small-sized, sensitive and selective. Microelectrodes with a carbon tip are employed for detecting DA *in vivo* due to their sensitivity and fast response to DA molecule [11–14]. Nevertheless, the detection of DA is disturbed by AA and other neurotransmitters, which response to carbon electrode, too. Aptamers are nucleic ligands which can bind to their target molecules with high specificity and affinity. This makes aptamers a powerful tool of selective detection of analysts, applied widely in biosensing and diagnostics [9, 15–19]. Herein, we developed a sensitive Electrochemical DNA (E-DNA) micro apta-sensor based on a nanometer-sized gold flower microelectrode (NGFME) we developed recently [20]. E-DNA sensors were developed based on the conformation changes of DNA and turned out to be highly sensitive and selective [21–23]. This micro E-DNA was capable of selectively detecting DA under the interference of ascorbic acid due to the high specificity of aptamer. This sensor is easy-to-prepare and has great potential to achieve DA detection in microenvironment.

II. EXPERIMENTAL

A. Apparatus and materials

Carbon fiber (Φ7 μm, Toray Industries Co., Ltd. Japan), glass capillary (Φ1 mm) and cooper wire (Φ0.5 mm) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Graphite powder conductive paint (Ted Pella, USA), DA, AA, $HAuCl_4$, Tris(2-carboxyethyl) phosphine (TCEP) and 6-Mercaptohexanol (MCH) were purchased from Sigma-Aldrich (St. Louis, MO). Cathodic electrophoresis paint (CEP) was obtained from Aomei Chemical Co., Ltd. (Shanghai, China). The DNA sequence (P1) in the experiment is 5'-SH-$(CH_2)_6$-GTCTCTGTGTGCGCCAGAGACACTGGGGCAGATATG GGCCAGCACAGAATGAGGCCC-MB-3' (Sangon Biotech Co., Ltd., Shanghai, China). All solutions were prepared with ultrapure water.

B. Preparation of carbon fiber microelectrode

The carbon fiber microelectrode (CFME) was prepared following the protocol developed by Huang *et al.* [24]. The carbon fiber was cleaned by sonication for 5 min successively in acetone, alcohol and ultrapure water, and dried at 37 °C. It was stuck onto a 10-cm cooper wire with graphite powder conductive paint and inserted into the glass capillary tip with an inner diameter of 20 μm, with an exposed tip of about

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0.5 mm. The glass capillary was sealed using an alcohol lamp. Finally, the tip of carbon fiber was etched to ∼200 µm length and ∼500 nm diameter by flame.

C. Fabrication of nano-flower gold microelectrode

The prepared CFME was encapsulated by CEP. The CEP solution was prepared by diluting CEP with water (1 : 5, by mass). The CFME tip was cleaned by sonication gently in ultrapure water and fully immersed in the CEP solution. In the deposition process of CEP, the CFME was used as the working electrode, and a platinum electrode was used as the reference and counter electrode. For uniform deposition, the platinum electrode was bent into circular shape (∼Φ1.5 cm) and the CFME was located at the circle center. The deposition was carried out under the "i–t" mode, with an initial potential of −8 V. After 100 s, the encapsulated CFME was washed with ultrapure water, heated to 80 °C and kept for 5 min. The CEP would be solidified and shrunk. A small area of CFME would be exposed. The apparent area of the exposed CFME was calculated from the steady current detected in 10 mmol/L $K_3[Fe(CN)_6]$ (containing 0.5 mol/L KCl) using Eq. (1),

$$i_0 = 2\pi nFD_c r_0, \tag{1}$$

where i_0 is the steady state current detected in 10 mmol/L $K_3[Fe(CN)_6]$, r_0 is the effective radius of the exposed tip of CFME, n is the electron transfer number in the oxidation-reduction reaction, D_c is the diffuse coefficient of the agent (7.2×10^{-6} cm^2/s for $K_3[Fe(CN)_6]$), and F is Faraday constant.

The encapsulated CFME of a similar size was selected in the experiment (∼250 nm in radius) and immersed in a $HAuCl_4$ solution containing 20 mmol/L $HAuCl_4$ and 0.5 mol/L HCl. Au deposition was carried out at −8 V for 100 s using DC potential amperometry in a three-electrode system with Ag/AgCl serving as a reference electrode.

D. Electrochemical detection of DA of NGFME

The prepared NGFME was allowed to incubate with 25 nmol/L P1 loading buffer (containing 1 mol/L NaCl, 20 mmol/L $MgCl_2$, 20 mmol/L PB and 3 mM TCEP) overnight in the dark. After that, the microelectrode was treated with 2 mmol/L MCH to tune the loading density of P1. The DA was detected in a solution containing 100 mM NaCl, 2 mM $MgCl_2$ and 20 mM PB, using a three-electrode system with "AC Voltammetry (ACV)" mode on the electrochemical station. Note that the solution of DA and AA should be prepared fresh at the time of use to prevent oxidation of the reagents.

E. Electrochemical detection of DA and AA of CFME

DA and AA detection of CFME was conducted in the detection solution containing 100 mM NaCl, 2 mM $MgCl_2$ and 20 mM PB (pH 7.4) using a three-electrode system through ACV mode on the electrochemical station.

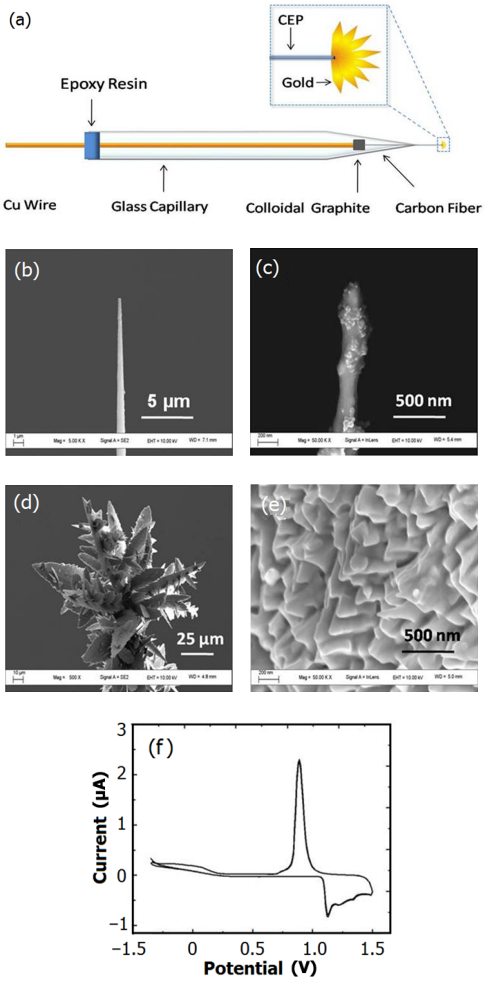

Fig. 1. (Color online) Schematics of a NGFME (a); Scanning electron microscope (SEM) images of the tip of CFME (b); CEP-encapsulated CFME (c); NGFME (d) and structure of the NGFME (e); and cyclic voltammetry curve (4 segments) of the NGFME measured in 0.05 mol/L H_2SO_4 (f).

III. RESULTS AND DISCUSSION

A. Fabrication and characterization of NGFME

The process of NGFME fabrication is illustrated in Fig. 1(a). Scanning electron microscope (SEM) images of the microelectrode tip are shown in Figs. 1(b)–1(e). Figure 1(b) is a general view of the CFME tip, revealing its smooth needle-like shape. After encapsulated by CEP, the tip surface became rugged (Fig. 1(c)). Figure 1(d) shows the NGFME tip, with flower-like structure after Au deposition. Cyclic voltammetry (CV) curves tested in 0.05 mol/L H_2SO_4 demonstrate the component of the flower-like structure is Au, with the special reductive peak of Au at 0.863 V (Fig. 1(f)). The structure is

sized at about 100 μm. Figure 1(e) shows that surface of the flower-like structure is spiny with thorns in nanometer level, with its surface area being comparable to a macroscopic gold electrode of 2-mm diameter.

B. The fabrication of micro E-DNA aptasensor

A novel E-DNA micro aptasensor was fabricated based on the NGFME for DA detection. As shown in Fig. 2(a), DA aptamer was modified with thiol motif at 5' and Methylene (MB) molecule at 3' end. The aptamer was assembled on surface of the NGFME through Au-S bonding. While DA was absent in the detection system, the presence of extended single-strand DNA kept MB away from the NGFME and hindered the electron transfer between MB and the gold surface. Therefore, ACV signal of the specific reducing peak of MB at -0.28 V was low (black curve in Fig. 2(b)). While DA was present in the system, DA would bind to its aptamer and cause the formation of secondary structure (Fig. 2(a)), hence the enhanced electron transfer between MB and gold and the increase of -0.28 V ACV signal (red curve in Fig. 2(b)). Therefore, DA detection can be achieved by this micro E-DNA sensor.

C. The optimization of loading concentration of DA aptamer

Sensitivity of the E-DNA sensor is related directly with loading density of the probe on the sensor surface [25]. High loading density shall hinder the aptamer folding and increase the background signal. With a low loading density of aptamer, the signal increase caused by the aptamer folding shall not be obvious. Thus, the concentration of loading concentration of P1 was optimized. We defined the signal increase of current at -0.28 V as "signal increase%" = $[(I_t - I_0)/I_0] \times 100\%$, where I_t and I_0 were the current at -0.28 V in presence and absence of DA, respectively. The DA aptamer concentration in loading buffer were 5–1000 nmol/L. From Fig. 2(c), in presence of 1 mM DA, the signal increase is the highest at DA aptamer concentration of 25 nM, which was chosen as the loading concentration of DA apatemer.

D. DA detection of the CFME and micro E-DNA sensor in the interference of AA

Microelectrodes with carbon tip were always employed for monitoring neurotransmitter release from cell [11, 13, 14]. The carbon microelectrode is sensitive and fast responding to DA, but not selective. We tested the response of 1 mM DA in the interference of 1 mM AA of CFME. As shown in Fig. 3(a), both the DA- and AA-generated oxidation curves on CFME and the peaks overlapped with each other. The peak current detected from the mixture of DA and AA was about 2 folds higher than that from DA only. Therefore, selective detection of DA cannot be achieved at the coexistence of AA

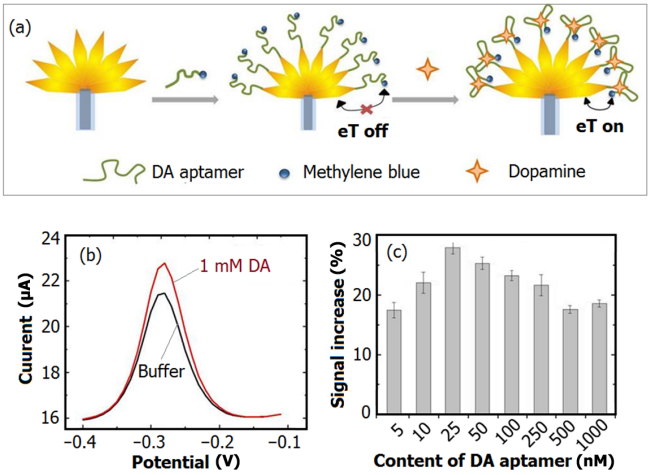

Fig. 2. (Color online) Schematic illustration (a) of the fabrication of micro E-DNA sensor based on NGFME; ACV signals (b) of the micro E-DNA sensor before and after reaction with 1 mM DA; and the "Signal Increase %" response (c) of the micro E-DNA sensor to different concentration of DA aptamer containing in loading buffer.

by CFME. After modifying the CFME with Au deposition and DA aptamer, the electrode responded selectively to DA. As shown in Fig. 3(b), no obvious signal increase response was observed at high concentration of AA (10 mM). What is more, the coexistence of AA with DA did not disturb the detection of DA, either. The result showed great potential for the detection of DA *in vivo*. The high selectivity of the micro E-DNA sensor was contributed to the high affinity between DA and its aptamer [9, 26, 27] (with the dissociation constant $K_d = 0.7$ μM in solution [26]).

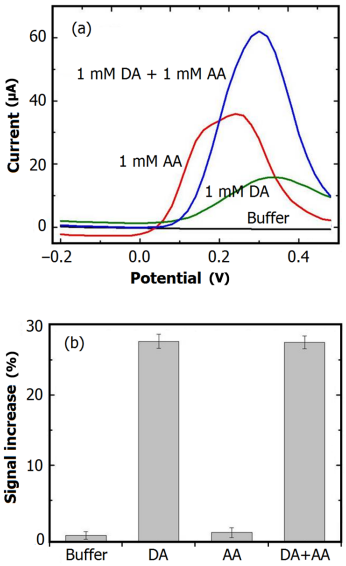

Fig. 3. (Color online) Electrochemical behavior (a) of DA and AA at CFME without modification; and comparison (b) of "signal increase%" of the micro E-DNA sensor by incubating with DA and AA. The concentration of DA and AA were 1 mM and 10 mM, respectively.

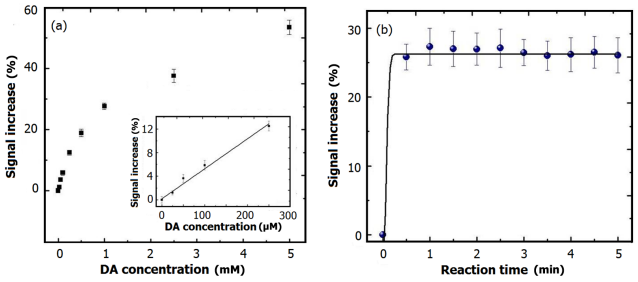

Fig. 4. Signal increase of the micro E-DNA sensor for target DA in the coexistence of 10 mM AA, as function of (a) DA content and (b) reaction time. The inset in (a) is the amplification of liner range from 25 to 250 μM. The data in (b) were taken every 30 s.

E. Quantitative detection of DA and the response time

To test the quantitative detection capability of the sensor in the interference of AA, calibration curve of the assay was obtained at different DA concentrations from 25 μM to 5 mM in the coexistence of 10 mM AA. As shown in Fig. 4(a). The signal increased with the DA concentration. A linear response was obtained in 25–250 μM ranges and the linear equation was "signal increase%" = $0.05092c_{\mathrm{DA}}$ (μM) + 0.1677 with a correlation coefficient of 0.9575. The detection limit of DA was 25 μM. The result illustrated that quantitative and selective detection of DA can be realized even the concentration of AA is several hundred folds higher than that of DA.

The response time of this micro E-DNA sensor to DA was tested through ACV measurement every 30 s. As shown in Fig. 4(b), this micro sensor responded rapidly to DA and exhibited near saturated signaling within 30 s of the addition of DA. The fast response of the sensor showed the enormous potential in real-time application.

IV. CONCLUSION

In summary, we have described a novel micro E-DNA aptasensor based on NGFME for DA detection, which processes several advantages. First, the sensor showed high sensitivity to DA even in the coexistence of high concentration AA, which was unable to be achieved by electrodes with a carbon tip. This property makes the micro E-DNA sensor be a promising system in *in vivo* detection. Second, the micro E-DNA sensor was fabricated on the basis of NGFME, which was cost-effective and easy-to-operate. The small size of the NGFME indicated that the sensor was able to be applied in microenvironment. Third, the response of the sensor to DA was very rapid, within 30 s. The fast response time allowed the real-time detection of DA. This novel sensing assay held a promise for real-time DA detection *in vivo* and in microenvironment with high specificity.

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